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Properties and strains of additional DNA repair-defective mutants in known and new genes of Neurospora crassa.

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Abstract

Some time ago, a number of mutants hypersensitive to MMS (methyl methane-sulfonate) were induced in Neurospora to obtain further types of DNA repair-deficient mutants; e.g., rec^- types not yet identified in Neurospora (meiotic-defective mutants generally are "hyperrec"; Schroeder 1986 Curr. Genet. 10:381-387).

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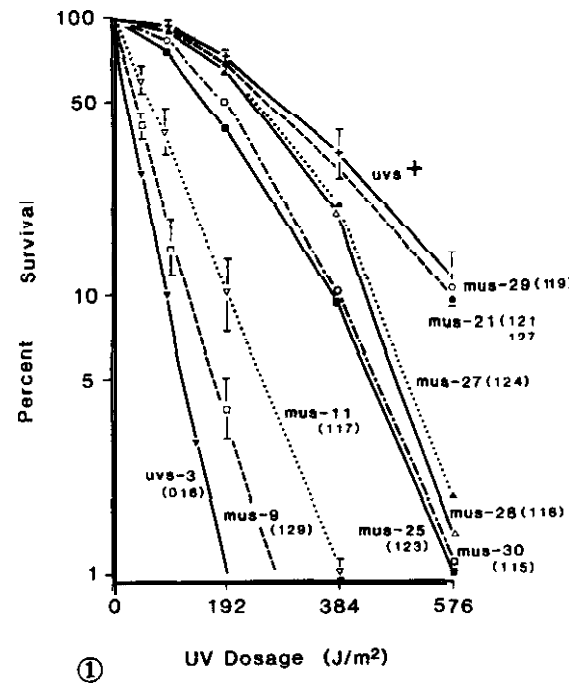
Most of the mutants listed in Tables 1 and 2 have been induced by 4NQ0 (4-nitroquinoline-l-oxide) as described for Aspergillus by Bal et al. (1977 Mutat. Res. 56:153-156). More recently, 3 further cases, mus (131 to 133), were isolated after UV-treatment. In both cases, a sn cr-1 strain (FGSC 4159) was mutagenized and the resulting colonies were tested on MMS (0.01% and 0.03%) and HIS (1 and 3 mg/ml) using velveteen replication. All mutants were backcrossed 2-4 times to wild type (74-ORS-lVA, FGSC 2489) as indicated in Table 2. The same wild type was also used as the control strains for all tests of hypersensitivity.

Genetic characterization of mus started usually with complementation tests in heterokaryons, using first prototroph strains on MMS-media and subsequently forced heterokaryons for confirmation, especially of negative results (Käfer and Perlmutter 1980 Can. J. Cytol. 22:535-552). Non-complementing pairs were further checked for recombination in intercrosses. However, since the latter were often sterile, most mutants were simultaneously crossed to alcoy; csp-2 (FGSC 3434) and mapped further to linked markers (Table 1). They were routinely checked for fertility in homozygous crosses and intercrossed to all available mus or uvs located on the two chromosomes involved in the linked alcoy translocation.

Tests for hypersensitivities to MMS and HIS were semi-quantitative (as described for HIS, Käfer 1981 Mutat. Res. 80:43-64) and UV survival was determined in several experiments (Figure 1). The obtained results permit a rough comparison between new and "old" alleles. and also between mutants in different genes. For example, the new allele mus(FK129) of mus-9 more closely resembles mus(FK109) than mus(FK104) (Table 1). The latter appears to be more defective than the other two and may well be a null allele. Furthermore, for MMS and HIS sensitivity, mus(FK133) resembles mus-21(SC10) while (FK131) and (FK132) are somewhat less sensitive.. However, their UV survival is about normal, similar to that of mei-2 (Schroeder and 01son 1983 Can. J. Genet. Cytol. 25:16-25).



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Five new genes are clearly identified by the various tests, namely mus-21, and mus-27 to -30 (Table 1). Five other cases which are included at the end of that table may also represent new genes. These mutants, mus(FK125) mus(FK128), and mus(FK131 to 133) need further backcrossing, and both (FK124 and (FK128) have shown certain problems. mus(FK125) produces very low and highly variable survival of conidia (0-5 to 40% when plated on standard minimal media and is unlinked to all mus and many other markers on linkage groups IV and V (in the absence of translocations). The mutant mus(FK128), on the other hand, produces two types of progeny in backcrosses which show different survival on MMS. It is uncertain whether is this related to the inability to identify linkage to alcoy; csp-2 (Table 1), but attempts to separate out two different mutants have so far been unsuccessful.

Table 1 Genetic Properties and cross-sensitivities of new *mus*

Results relative to mus^+ control: 0 - like wild type;
1.5x, 8x, etc., - dose modifying factors; ±, +, ++ - increased levels of sensitivities:
NT - not tested; ? - preliminary results

mus Gene - allele numbers	Linkages Group & arm	Linked marker[a]	Fertility: homozygous crosses	Approx. conidial viability	Sensitivities to mutagens and inhibitors MMS	HIS	MC	uv
1) Alleles of known genes								
-7 (FK116)	IIR	nuc-2	barren	100%	>8x	>100x	NT	NT
(FK107)^b		p:12%	barren	100%	>8x	>200x	±	0
-9 (FK129)	IR	al-2	sterile	80%	>8x	>10x	+	4x
(FK104)^b		p:6%	sterile	25%	>8x	>100x	+	5-6x
(FK109)^b			sterile	75%	>8x	>50x	+	5-6x
-11 (FK117)	VR	pab-2	barren	75%	>10x	>100x	0	3x
(FKlll)^b		d:23%	barren	80%	>10x	>200x	+	2x
-25 (FK123)^c	VIIR	met-7 d:6%	barren	90%	6x	20x	±	>1.5x
2) Mutants in new genes								
-21 (FK121)	IIIR	trp-1	barren	100%	6x	5x	0	0
(FK120)		d:20%	barren	80%	6x	>5x	NT	NT
(FK127)			barren	90%	>6x	2x	NT	0
(SC10)^d			±sterile	80%	>6x	>15x	NT	2x
-27 (FK124)	IIR	nuc-2 d:16%	fertile	85%	>8x	50x	0	<1.5x
-28 (FK118)	VL	lys-1 d:10%	fertile	100%	2x	>2x	0	<1.5x
-29 (FK119)	VIL	cho-2 p:14%	sterile	50%	2x	20x	0	0
-30 (FK115)	IVR	trp-4 p:4%	fertile	80%	>4x	2x	0	>1.5x
3) Unmapped new *mus*								
mus(FK125)	VL?	Alcoy cot T(IV;V) 32%	fertile	2-20%	3x	>20x	NT	±
mus(FK128)	NT	Alcoy unlinked	barren	85%	4x	>5x	0	0
mus(FK131)	Complementing all *mus* listed above, *mus-8*, *mus-10*, all MMS. sensitive *uvs* and *mei-2*.		NT	80%	>3x	15x	NT	0
mus(FK132)			NT	75%	>3x	15X	NT	0
mus(FK133)			NT	85%	5x	20x	NT	0

^a p or d = proximal or distal to linked marker indicated.
^b Compared to published cases (Käfer 1981. ref. cit.).
^c Allelic with *mus-25*(SA3) (Inoue and Ishii 1984 Mutat. Res. 125:185-194 and pers. comm.).
^d Mutant of DeLange and Mishra (1982 Mutat. Res. 96:187-199).

All these mutants are being deposited at FGSC (Table 2) and additional detailed information will be made available to anyone interested in analyzing them further (their investigation is being discontinued).

Table 2. New mus strains available from FGSC

mus Gene	Allele	Prototroph Level of backcross		FGSC no. A	a	Simple Marker gene	requiring allele	FGSC no. A	a
mus-7	FK116	II(A)	I(a)	6401	6402	--	--	--	--
mus-9	FK129	IV		6403	6404	leu-3	R156	6405	--
						nic-2	43002	6407	6408
mus-11	FK117	IV		6409	6410	pan-l	5531	6411	6412
						lys-1	33933	6413	--
mus-21	FK121	VI		6414	6415	trp-1	10575	6416	6417
	FK120	II		6418	6419	trp-1 acr-2	10575 KH5	6420	6421
	FK127	II		6422	6423			--	--
mus-25	FK123	IV		6424	6425	nic-3	Y31881	6426	6427
mu-27	FK124	IV		6428	6429	arg-5	27947	6430	6431
						nuc-2	T28M2	6432	6433
mus-28	FK118	IV		6434	6435	leu-1	33757	6436	6437
mus-29	FK119	IV		6438	6439	trp-2	41	6440	6441
					ylo-1	pan-2	Y30539y Y153M96	6442	6443
mus-30	FK115	IV		6444	6445	pan-l	5531	6446	6447
						met-2	K43	6448	6449
mus(FK125)		IV		6450	6451	rib-l	51602(t)	6452	6453
						pan-l	5531	6454	6455
mus(FK128;		IV		--	6457	lys-5	DS6-85	6458	--
mus(FK131)		II		6459	6460	trp-2	41	6461	6462
mus(FK132)		II		6463	6464	rib-l	51602(t)	6465	6466
mus(FK133)		II		6467	6468	met-1	M105	6469	6470

Tests of mus(FK115), (FK119) and (FK123) for allelism to recently mapped genes by Dr. H. Inoue are gratefully acknowledged. This work was supported by NSERC of Canada.
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